Food Chemistry 112 (2009) 982–986

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The bioavailability of iron fortified in whole grain parboiled rice

Chanakan Prom-u-thai [a,c,*], Raymond P. Glahn [b], Zhiqiang Cheng [b], Shu Fukai [a], Benjavan Rerkasem [c], Longbin Huang [d]

[a] School of Land, Crop and Food Science, University of Queensland, St. Lucia, QLD 4072, Australia
[b] The Robert W. Holly Center for Health and Agriculture, Agricultural Research Service, US Department of Agriculture, Tower Road, Ithaca, NY, 14853, USA
[c] Agronomy Department, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[d] Center for Mined Land Rehabilitation, University of Queensland, St. Lucia, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 8 April 2008
Received in revised form 16 May 2008
Accepted 9 July 2008

Keywords:
Parboiled rice
Bioavailability
Fe uptake
Caco-2 cell
Fortified rice

ABSTRACT

The present study was to evaluate the bioavailability of iron (Fe) fortified in parboiled rice grain, expressed as Fe uptake by Caco-2 cells after *in vitro* digestion. The bioavailability of Fe-fortified in the rice grain was closely and positively correlated with increasing concentrations of Fe in the grains of the three cultivars ($r = 0.96^{**}$). The uptakes of the Fe-fortified in parboiled rice milled for 120 s (34.2, 47.7 and 107 ng ferritin mg protein^{-1} in three cultivars, respectively) were well above those of the unfortified raw (6.1, 4.9 and 5.7 ng ferritin mg protein^{-1}) or parboiled rice (4.7, 3.6 and 4.4 ng ferritin mg protein^{-1}), the high Fe rice line IR68144-2B-3-2-2 (4.0 ng ferritin mg protein^{-1}) and popular Jasmine rice cultivar KDML 105 (3.9 ng ferritin mg protein^{-1}). Increasing milling time and rinsing the Fe-fortified parboiled rice decreased Fe bioavailability, due to their negative effects on total Fe concentrations in the parboiled rice grains, but uptakes were still well above that of their unfortified raw or parboiled rice grains. Rinsing or washing the Fe-fortified and milled rice grains decreased the bioavailability to 85 ng ferritin mg protein^{-1} in the YRF cultivar, compared to about 100 ng ferritin mg protein^{-1} in its non-rinsed grains. Dilute acid-extractable (DAE) Fe was linearly, positively correlated with the uptake of Fe assessed by the *in vitro* digestion/Caco-2 cell technique ($r = 0.90^{**}$), which can be used as a rapid method for optimizing levels of bioavailable Fe to be fortified in the parboiled rice by parboiled-rice mills if this Fe-fortification technique should be adopted in south and southeast Asia.

1. Introduction

Iron deficiency is the most common nutritional disorder in the world, causing severe health impacts and economic losses, particularly among populations suffering economic disadvantages and limited access to Fe-rich food ingredients in Asia and Africa (Bouis, 1996; Welch & Graham, 2004). It is estimated to affect more than 3.6 billion people in the developing world (World Health Organization, 2002). Both short-term and long-term solutions have been researched and applied to address this problem, including direct Fe fortification in food (such as rice and wheat flours) and biofortification by selecting and breeding crops of high Fe density (Graham & Welch, 1996; Hurrell, 1998; Welch & Graham, 2002). Our research group has recently pioneered an innovative approach to fortify Fe in parboiled rice as a short-term solution to Fe deficiency in south Asia and South Africa where there are concentrated regions of parboiled rice consumption (Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008).

Parboiled rice is made from approximately 20–50% of world rice production and is the staple food in South Asian countries and Africa (Bhattacharya, 2004; Choudhury, 1991; Pillaiyar, 1981). Iron concentrations in Fe fortified parboiled rice grains (white rice) were increased to as high as 70–144 mg Fe kg^{-1} dry weight in 60 s milled parboiled rice (Prom-u-thai et al., 2008), compared to the relatively low Fe status in raw rice grains from conventional breeding (7–13 mg Fe kg^{-1} dry weight) and 37 mg Fe kg^{-1} dry weight from transgenic rice (Vasconcelos et al., 2003). These findings about the significant enhancement of Fe density in parboiled rice through Fe-fortification provide a basis for developing a potentially cost-effective and highly adoptable solution to the improvement of Fe intake in the rice-based diet of these countries, by taking advantage of the production process already widely used in the industry.

However, the bioavailability of fortified Fe in the parboiled rice has not been assessed, nor have various factors which are most likely to affect its bioavailability. This information will provide an important basis for further research establishing criteria for optimizing Fe density and uptake of the fortified Fe in parboiled rice.

* Corresponding author.Address: School of Land, Crop and Food Science, University of Queensland, St. Lucia, QLD 4072, Australia. Tel.: +61 7 33652526; fax: +61 7 33651177.

E-mail addresses: chanakan@uq.edu.au, chanakan15@hotmail.com (C. Prom-u-thai).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.07.020

Our initial assessment showed that 60–95% of Fe in the milled grain of Fe-fortified-parboiled rice is in the DAE Fe form (free Fe) (Prom-u-thai et al., 2008), indicating that the fortified Fe in parboiled rice is potentially highly bioavailable Fe for human uptake.

The objectives of the present study were: (1) To investigate the relationship between Fe concentrations in the fortified parboiled rice and the uptake of the Fe quantified by an *in vitro* digestion/Caco-2 cell technique (Glahn, Chen, Welch, & Gregorio, 2002; Glahn, Lee, Yeung, Goldman, & Miller, 1998), for the estimation of the relative efficacy of fortified Fe in the parboiled rice. (2) To establish the relationship between DAE Fe and uptake of Fe as an indirect bioavailable Fe index for developing a rapid and reliable method of estimating bioavailable Fe density in rice grains, to be used in parboiled-rice mills for routine analysis. (3) To measure the effect of rinsing and milling time on Fe uptake. For comparison, an advanced line for high Fe concentration from IRRI, IR68144-2B-3-2-2, a popular Thai Jasmine rice, KDML 105, a commercial Thai parboiled rice and a commercial white bean from the USA (rich in bioavailable Fe) have been used as references in the assessment of uptake of fortified Fe in the parboiled and milled rice grains.

The *in vitro* digestion/Caco-2 cell technique has been confirmed as the best *in vitro* technique to assess the relative amount of bioavailable Fe in foods, and is useful under conditions where screening is necessary to refine the test samples for *in vivo* trials (Ariza-Nieto, Blair, Welch, & Glahn, 2007; Oikeh, Menkir, Maziya-Dixon, Welch, & Glahn, 2003, 2004). Iron uptake in this model is measured via the formation of ferritin - the intracellular Fe storage protein, which forms in response to cell-iron uptake in this intestinal epithelial cell line (Glahn et al., 1998, 2002). This technique has previously been applied to measure the bioavailability of Fe in rice samples (Glahn et al., 2002; Prom-u-thai et al., 2006).

2. Materials and methods

2.1. Fe-fortified-parboiled rice

Samples of Fe-fortified-parboiled and unfortified raw and parboiled rice of 3 cultivars (Echuga, YRF 2 and Opus) were selected from a previous study (Prom-u-thai et al., 2008). The total Fe concentration of rice samples selected from the previous experiment are shown in Table 1. The Fe fortification process has been described previously (Prom-u-thai et al., 2008). Briefly, 150 g of paddy rice were subsampled for each cultivar and rinsed thoroughly in 3 changes of distilled deionised water (DDI) before applying treatments. For Fe fortification treatments, rinsed paddy rice was soaked with 150 ml of Fe solution, consisting of ferrous sulphate ($FeSO_4$) and ethylenediaminetetra-acetic acid disodium salt (Na_2EDTA) mixed in 2:1 molar ratios with 11.16 g Fe per 100 g of paddy rice (~13% moisture content). For producing the unfortified parboiled rice (control), rinsed paddy rice was soaked with 150 ml of distilled triple deionised water (TDI) for 24 h at room temperature. Soaked grains, after draining off free water, were steamed with a pressure of 2.29 kg cm^{-2} at 110 °C for 10 min. The grains were cooled and sun-dried to approximately 11% moisture content.

2.2. Husking and milling

The preparation of brown and white rice of the parboiled rice was described in Prom-u-thai et al. (2008). The dried parboiled rice grains were separated into brown rice (unmilled caryopsis) and husk (palea and lemma) with a testing husker (Satake model THU-35A, Japan), followed by milling for 60 or 120 seconds to yield white rice, using a laboratory milling machine (Satake model TM 05, Japan). Aliquots of the grain samples (unmilled and milled) were digested in nitric-perchloric acid (5:1) at 120–180 °C and analyzed for total Fe concentration by ICP-AES (Zarcinas, Cartwright, & Spouncer, 1987).

2.3. Rinsing of samples

Samples were rinsed to simulate rice washing behaviour when cooking parboiled rice. The milled samples of raw, non-fortified and Fe-fortified parboiled rice were thoroughly rinsed in 3 changes of 200 ml of TDI water (2:1 v/w (water/rice) ratio) (Hettiarachchi, Hilmers, Liyanage, & Abrams, 2004; Tulyathan, Mekjarutkul, & Jongkaewwattana, 2005) and after being drained of water, autoclaved for 15 min to simulate the cooking process for preparing cooked rice samples. The cooked rice samples were then homogenized in a Polytron homogenizer and the homogenate was frozen and then lyophilized to dryness before testing via the *in vitro* digestion/Caco-2 cell system.

2.4. Dilute acid-extractable Fe

For dilute acid extraction, approximately 0.5 g of milled Fe-fortified-parboiled rice grains was weighed into a 50 ml flask. Each sample was treated with 10 ml of 0.1 M HCl and placed on a hotplate at 60 °C for 30 min. After the extraction of soluble Fe, the residue was analyzed for insoluble Fe by the method described in total Fe analysis. Soluble Fe in the fortified Fe rice was calculated by the deduction from total Fe concentration of insoluble Fe concentration.

Table 1
Fe concentration in milled rice of Fe-fortified-parboiled rice and unfortified raw and parboiled rice after milling for 60 and 120 s of 3 cultivars

Cultivar	Milling time (seconds)	Fe concentration (mg kg^{-1})		
		Unfortified		Fe-fortified-parboiled
		Raw	Parboiled	
Echuga	60	5.2 ± 0.6	4.5 ± 0.4	68.1 ± 3.4
	120	3.6 ± 0.6	3.0 ± 0.3	27.7 ± 0.3
YRF 2	60	3.7 ± 0.8	3.5 ± 0.8	82.9 ± 6.8
	120	2.0 ± 0.5	2.0 ± 0.7	41.8 ± 5.0
Opus	60	7.2 ± 0.3	8.4 ± 0.9	144 ± 29.3
	120	2.2 ± 0.1	3.6 ± 0.1	110 ± 3.6
IR68144-3B-2-2-3[a]		3.6 ± 0.3	–	–
KDML 105[a]		2.9 ± 0.6	–	–
Commercial Thai parboiled rice[a]		2.2 ± 0.1	3.4 ± 0.2	
Commercial white bean[a]		58.0	–	–

Values are means ± SEM (*n* = 3) (Prom-u-thai et al., 2008).
[a] Origin of line IR68144-3B-2-2-3 is IRRI, Philippines. KDML 105 and commercial Thai parboiled rice is from Thailand. Commercial white bean is from USA.

2.5. Bioavailable Fe

Iron uptake from the rice samples was measured via an *in vitro* digestion of 1 g cooked rice samples and subsequent exposure of that digest to cultured Caco-2 cells, thus simulating Fe uptake at the intestinal surface. In this model, Caco-2 cells' ferritin formation was used as a proxy for Fe uptake by the enterocytes (Glahn et al., 1998). Immunoradiometric assay was used to quantify Caco-2 cell ferritin content (FER-Iron II Ferritin Assay, RAMCO Laboratories, Houston, TX). A 10 μl sample of the sonicated Caco-2 cell monolayer; harvested in 2 ml of water, was used for each ferritin measurement (Glahn et al., 1998). The $FeCl_3$ with ascorbic acid (50 μM Fe with 1 mM ascorbic acid) was used as a positive control to verify responsiveness of the Caco-2 cells to uptake of Fe. An advanced line, with improved Fe concentration from IRRI, IR68144-3B-2-2-3, a popular Jasmine rice from Thailand, KDML105, a commercial Thai parboiled rice and a commercial white bean (rich in bioavailable Fe), was included in the assay of Fe uptake for the purpose of comparison with the Fe-fortified parboiled rice.

2.6. Data analysis

Analysis of variance was carried out to detect the differences of Fe uptake between Fe-fortified-parboiled rice and unfortified raw and parboiled rice, under different milling time and rinsing conditions of 3 rice cultivars by using Statistic 7, analytical software, SXW (Tallahassee, FL, USA). The least significant difference (LSD) at $P< 0.05$ was applied to compare the means for any significant difference. The correlation coefficients between the uptake of Fe and Fe concentration and between uptake of Fe and Fe solubility in dilute acid extraction were determined in Fe-fortified-parboiled rice of 3 cultivars.

3. Results

The uptake of Fe by Caco-2 cells, in the 120 s (equivalent commercial milling time) milled grains, increased significantly with increasing levels of Fe loaded into the parboiled rice (Fig. 1). The uptake of Fe in Fe-fortified-parboiled rice ranged from 34 to 107 ng ferritin mg protein^{-1} across the 3 cultivars tested. In comparison, the unfortified raw or parboiled rice of these cultivars had very low levels of Fe uptake, 4–7 ng ferritin mg protein^{-1}. IR68144-2B-3-2-2 (a selected high Fe line) and KDML 105 (a popular jasmine rice) selected from conventional breeding had the lowest level of bioavailable Fe, less than 5 ng ferritin mg protein^{-1}. Commercial US white bean, as a reference sample, which is known to be rich in bioavailable Fe, had about 57 ng ferritin mg protein^{-1}. The cultivar Opus (107 ng ferritin mg protein^{-1}) had a higher Fe uptake than had YRF 2 (47.7 ng ferritin mg protein^{-1}) or Echuga (34.2 ng ferritin mg protein^{-1}), which matched the order of the fortified Fe density in their parboiled rice grains (Fig. 1).

There was a close positive correlation between Fe uptake and total Fe concentrations in the rice samples tested: the unfortified raw and parboiled rice and Fe-fortified parboiled rice of the 3 cultivars tested ($r = 0.96^{**}$) (Fig. 2).

Increasing milling time decreased the level of Fe loaded into the Fe-fortified parboiled rice and its Fe uptake (Fig. 3, Table 1), but the Fe uptake remained relatively high at 35–120 ng ferritin mg protein^{-1} after milling for 120 s, which was well above those of the unfortified raw and parboiled rice grains and the high Fe rice, and remained comparable to that of the legume sample – commercial US white bean (Fig. 3, Table 1).

Rinsing the Fe-fortified, parboiled grains of YRF 2 decreased total Fe concentrations in the grains and thus the amount of bioavailable Fe in the grain, based on the Fe uptake test by Caco-2 cells

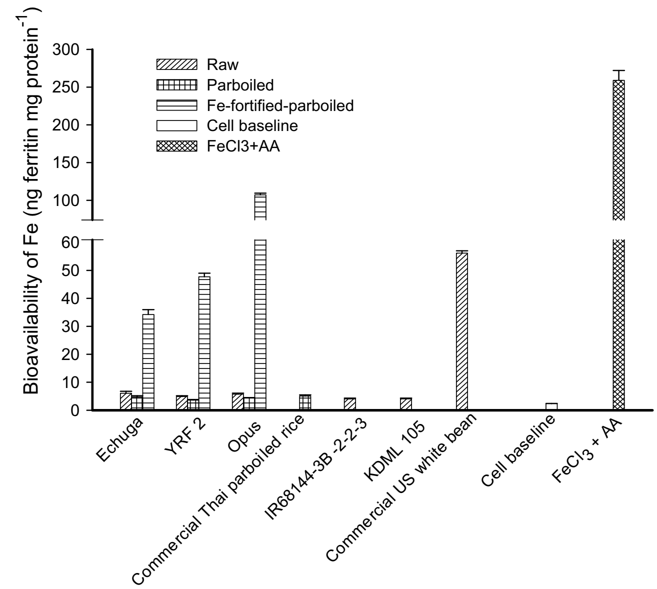

Fig. 1. Bioavailability of Fe from digests of rice samples in 3 cultivars after milling for 120 s. Commercial Thai parboiled rices, IR68144-2B-3-2-2, KDML 105 and a commercial US white bean are also included. Values are means ± SEM ($n = 3$). $FeCl_3$ with ascorbic acid (50 μM Fe with 1 mM ascorbic acid) served as a positive control to verify responsiveness of the Caco-2 cells to bioavailable Fe.

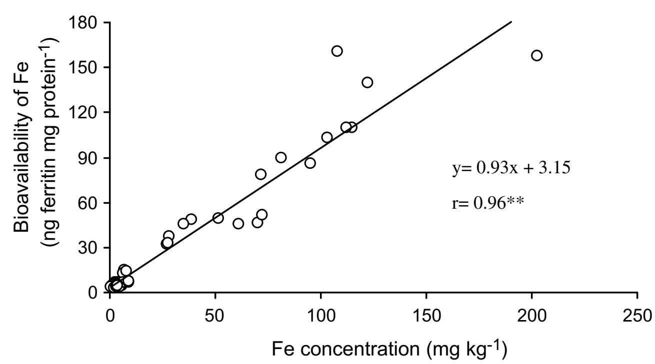

Fig. 2. Relationship between bioavailability of Fe and total Fe concentration in unfortified raw and parboiled rice and Fe-fortified-parboiled rice of the 3 cultivars. The dataset was from test results with grains milled for 60 and 120 s ($n = 54$).

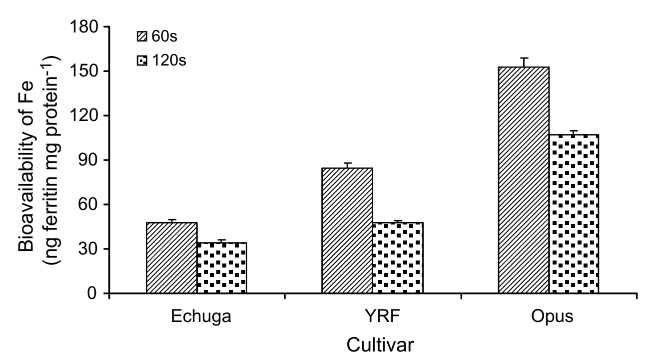

Fig. 3. Effect of milling time (60 and 120 s) on the bioavailability of Fe from raw, parboiled, and Fe-fortified parboiled rices of selected cultivars. Values are mean ± SEM ($n = 3$).

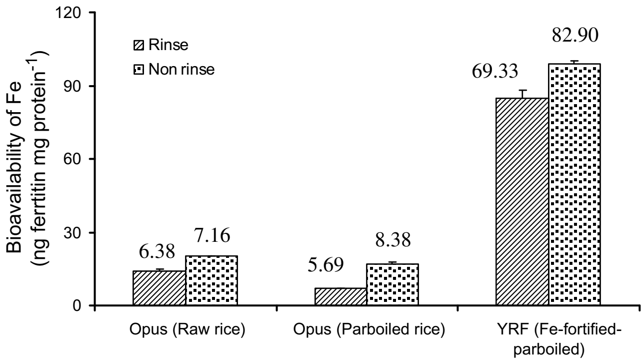

Fig. 4. Effects of rinsing before cooking on the bioavailability of Fe from raw, parboiled, and Fe-fortified parboiled rices of selected cultivars. The different types of grains were all milled for 60 s. Values are means ± SEM (n = 3). Numbers above the bar are Fe concentrations (mg kg^{-1}).

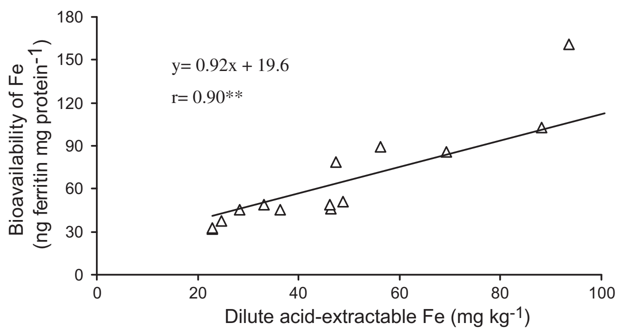

Fig. 5. Relationship between the dilute acid-extractable Fe and Fe bioavailability (*in vitro* test) in 3 cultivars of Fe-fortified-parboiled rice. The dataset was pooled from test results with grains milled for 60 and 120 s (n = 18).

(Fig. 4). However, this effect was not strong enough to diminish the enhanced levels of bioavailable Fe in the Fe-fortified parboiled grain, resulting in 85 ng ferritin mg $protein^{-1}$ Fe uptakes in rinsed grains, compared to about 100 ng ferritin mg $protein^{-1}$ of non-rinsed grains in cultivar YRF 2 (Fig. 4). This level of Fe uptake in rinsed grains remained well above those of unfortified raw and parboiled rice, remaining comparable to that in the reference sample of white bean. The rinsing effects were also observed in unfortified raw and parboiled rice grains in cultivar Opus (Fig. 4).

Moreover, the present study found that there was a close positive linear correlation between the uptake of Fe and DAE Fe in milled grain of Fe-fortified-parboiled rice grains of the 3 rice cultivars ($r = 0.90^{**}$) (Fig. 5).

4. Discussion

Our previous study has established that Fe fortification of paddy rice during the parboiling process increased the Fe concentrations in milled rice grain by about 20-50 times, compared to those in unfortified raw rice (Prom-u-thai et al., 2008). In the present study, the amount of bioavailable Fe, which was assessed using the *in vitro* digestion/Caco-2 cell culture technique, was closely and positively correlated with the increasing level of Fe-fortified in the parboiled rice grains, regardless of cultivars. This suggests a high efficacy potential of the Fe-fortified in the whole grain of parboiled rice for improving Fe nutrition in rice-based human diets. Increasing milling time and rinsing the Fe-fortified parboiled rice decreased the amount of bioavailable Fe, due to their negative effects on the total Fe concentrations in the parboiled rice grains; however, the values remained well above those in the unfortified raw or parboiled rice. This is because more than 50% of the fortified Fe in the fortified parboiled rice grains was retained in the grains after 3 repeated rinsings, except for the Opus variety milled for 120 s (Prom-u-thai et al., 2008). There was a close linear relationship between DAE Fe concentrations and the uptake of Fe assessed by the *in vitro* digestion/Caco-2 cell culture technique. As a result, the analysis of DAE Fe can be used as a rapid method for quantifying levels of potentially bioavailable Fe in the Fe-fortified parboiled rice in parboiled rice mills if this whole grain Fe-fortification technique should be adopted for use in south and southeast Asia. For comparison, the levels of bioavailable Fe in the Fe-fortified parboiled rice grains were well above the unfortified raw or parboiled rice of the improved line of high Fe rice IR68144-2B-3-2-2 and popular Thai Jasmine rice, KDML 105, and were even comparable to the Fe-rich legume - a commercial white bean.

The results strengthen the suggestion that Fe fortification of parboiled rice could be an effective solution for improving Fe nutrition in populations exposed to the risk of Fe deficiency from limited access to high Fe food. The fortified Fe in the parboiled rice remained highly bioavailable, even after 120 s milling or repeated rinsing treatments. This is because of the inward movement of Fe into the endosperm cell layers (Prom-u-thai et al., 2008). The increased distribution of the fortified Fe in the endosperm during the parboiling process is much higher than the natural distribution of Fe in unfortified raw or parboiled rice grains.

Fortification of Fe through the parboiling process seems to induce the movement of fortified Fe into the inner part of grain and endosperm, possibly via the dorsal vascular bundle present in the kernel (Prom-u-thai et al., 2008). The uptake of Fe in Fe-fortified-parboiled rice was about 8 or 22-fold higher than that in unfortified-raw and parboiled rice in 60 s milled grain and 5 or 24-fold in 120 s milled grain among the different rice varieties tested. Moreover, the uptake of Fe by Caco-2 cells was also higher in Fe-fortified-parboiled rice than in a commercial Thai parboiled rice, KDML 105, a popular Jasmine rice, IR68144-2B-3-2-2, an improved line with high Fe concentration and a commercial white bean consumed in the USA as a source of Fe nutrition. This suggests that Fe fortified in parboiled rice, even after milling to produce white rice, is in a highly bioavailable form that can help parboiled rice consumers.

The significantly elevated levels of bioavailable Fe in the Fe-fortified parboiled rice grains was also related to the high solubility of the fortified Fe retained in the grains. In the 3 cultivars tested, more than 60% of the fortified Fe remained soluble in dilute acid. The uptake of Fe in milled grain of Fe-fortified-parboiled rice is linearly correlated with DAE Fe ($r = 0.90^{**}$). This suggests that DAE Fe can be used as an indirect indicator of potentially bioavailable Fe in rice, in addition to the Fe uptake assayed by the *in vitro* digestion/Caco-2 cell culture technique. This will provide a simple and rapid method for assessing and optimizing the level of Fe fortification in parboiled rice of different cultivars when the Fe-fortification technique is adopted by the rice mills.

Iron fortification of parboiled rice have its advantages over direct food fortification with an alternative NaFeEDTA compound, such as in rice flour (Hettiarachchi et al., 2004), rice meal (Haas et al., 2005; MacPhail, Patel, Bothwell, & Lamparelli, 1994), corn (Walter, Pizarro, & Olivares, 2003) and fish/soy sauces (Fidler, Davidsson, Walczyk, & Hurrell, 2003; Thuy et al., 2003). Iron fortification of raw rice grains has been developed by special and expensive rice surface coating techniques such as the flour gel (Tulyathan et al., 2005) and cellulose polymer coated methods (Peil, Barrett, Rha, & Langer, 1981). However, it has not been successful in the market as consumers easily detect the altered colour and habitually remove the fortified grains mixed with the normal grains (Hurrell & Cook, 1990). Iron-fortification of parboiled rice can utilize the existing industrial infrastructure for processing parboiled rice and the well-established distribution network in developing countries, such as Bangladesh, Sri Lanka, India and South Africa, where parboiled rice is mostly being consumed (Choudhury, 1991). However, before any commercial adoption of this technology, the following questions have to be addressed satisfactorily in further research: (1) What is the sensory quality of the Fe-fortified parboiled rice? (2) Can the bioavailability of the fortified Fe decline over time under normal storage conditions? (3) What is the economically feasible loading rate of Fe for producing the Fe-fortified parboiled rice?

Acknowledgements

We would like to thank Dr Russell Reinke and all assistants at Yanco Agriculture Institute, NSW, Australia for their help in the field experiment and parboiled rice mill operators in central Thailand for their information on parboiled rice process and sample collections. Seeds of IR68144-2B-3-2-2 were provided by IRRI and KDML 105 by the Thai Rice Department.

References

Ariza-Nieto, M., Blair, M. W., Welch, R. M., & Glahn, R. P. (2007). Screening of iron bioavailability patterns in eight bean (Phaseolus *vulgaris* L) genotypes using the Caco-2 cell in vitro model. *Journal of Agriculture and Food Chemistry, 55*, 7950–7956.

Bhattacharya, K. R. (2004). Parboiling of Rice. In T. C. Elaine (Ed.), *Rice Chemistry and Technology* (3rd ed., pp. 329–404). St. Paul, Minnesota: American Association of Cereal Chemists, Inc.

Bouis, H. E. (1996). Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. *Nutrition Reviews, 54*, 131–137.

Choudhury, N. H., 1991. Parboiling and Consumer Demand of Parboiled Rice in South Asia. International Rice Research Institute, Manila, 47–54.

Fidler, M. C., Davidsson, L., Walczyk, T., & Hurrell, R. F. (2003). Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA. *American Journal of Clinical Nutrition, 78*, 274–278.

Glahn, R. P., Chen, S. Q., Welch, R. M., & Gregorio, G. B. (2002). Comparison of iron bioavailability from 15 rice genotypes: Studies using an in vitro digestion/caco-2 cell culture model. *Journal of Agricultural and Food Chemistry, 50*, 3586–3591.

Glahn, R. R., Lee, O. A., Yeung, A., Goldman, M. I., & Miller, D. D. (1998). Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *Journal of Nutrition, 128*, 1555–1561.

Graham, R. D., & Welch, R. M., 1996. Breeding for Staple-Food Crops with High Micronutrient Density. International Food Policy Research Institute, 1–72.

Haas, J. D., Beard, J. L., Murray-Kolb, L. E., del Mundo, A. M., Felix, A., & Gregorio, G. B. (2005). Iron-biofortified rice improves the iron stores of nonanemic Filipino women. *Journal of Nutrition, 135*, 2823–2830.

Hettiarachchi, M., Hilmers, D. C., Liyanage, C., & Abrams, S. A. (2004). Na(2)EDTA enhances the absorption of iron and zinc from fortified rice flour in Sri Lankan children. *Journal of Nutrition, 134*, 3031–3036.

Hurrell, R. F. (1998). Improvement of trace element status through food fortification: Technological, biological and health aspects. *Bibliotheca Nutrition et Dieta, 54*, 40–57.

Hurrell, R. F., & Cook, J. D. (1990). Strategies for iron fortification of foods. *Trends in Food Science & Technology, 1*, 56–61.

MacPhail, A. P., Patel, R. C., Bothwell, T. H., & Lamparelli, R. D. (1994). EDTA and the absorption of iron from food. *American Journal of Clinical Nutrition, 59*, 644–648.

Oikeh, S. O., Menkir, A., Maziya-Dixon, B., Welch, R. M., & Glahn, R. P. (2003). Assessment of concentrations of iron and zinc and bioavailable iron in grains of early-maturing tropical maize varieties. *Journal of Agricultural and Food Chemistry, 51*, 3688–3694.

Oikeh, S. O., Menkir, A., Maziya-Dixon, B., Welch, R. M., & Glahn, R. P. (2004). Assessment of iron bioavailability from twenty elite late-maturing tropical maize varieties using an in vitro digestion/Caco-2 cell model. *Journal of the Science of Food and Agriculture, 84*, 1202–1206.

Peil, A., Barrett, F., Rha, C., & Langer, R. (1981). Retention of micronutrients by polymer coatings used to fortify rice. *Journal of Food Science, 47*, 260–262.

Pillaiyar, P. (1981). Household parboiling of parboiled rice. *Kishan World, 8*, 20–21.

Prom-u-thai, C., Fukai, S., Godwin, I. D., Rerkasem, B., & Huang, L. (2008). Iron-fortified parboiled rice – A novel solution to high iron density in rice-based diets. *Journal of Food Chemistry, 110*, 390–398.

Prom-u-thai, C., Glahn, R. P., Welch, R. M., Fukai, S., Rerkasem, B., & Huang, L. (2006). Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes. *Journal of the Science of Food and Agriculture, 86*, 1209–1215.

Thuy, P. V., Berger, J., Davidsson, L., Khan, N. C., Lam, N. T., Cook, J. D., et al. (2003). Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. *American Journal of Clinical Nutrition, 78*, 284–290.

Tulyathan, V., Mekjarutkul, T., & Jongkaewwattana, S. (2005). Iron retention on flour gel-coated rice grains and its storage stability. *Foodservice Research International, 15*, 147–156.

Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torrizo, L., Krishnan, S., et al. (2003). Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Science, 164*, 371–378.

Walter, T., Pizarro, F., & Olivares, M. (2003). Iron Bioavailability in corn-masa tortillas is improved by the addition of disodium EDTA. *Journal of Nutrition, 133*, 3158–3161.

Welch, R. M., & Graham, R. D. (2002). Breeding crops for enhanced micronutrient content. *Plant and Soil, 245*, 205–214.

Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany, 55*, 353–364.

World Health Organization (2002). *The world health report 2002*. Geneva, Switzerland: World Health Organization.

Zarcinas, B. A., Cartwright, B., & Spouncer, L. R. (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communication of Soil Science and Plant Analysis, 18*, 131–146.